# Sulfuryl Fluoride as a Quarantine Treatment for Anoplophora glabripennis (Coleoptera: Cerambycidae) in Regulated Wood Packing Material

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ABSTRACT The Asian longhorned beetle, Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), was probably introduced into the United States from China with solid wood packing and dunnage during the 1980s, and it has recently become established in limited infestations near several major cities in the United States. Regulated wood packing material (RWPM) arriving in the United States from China is required to undergo fumigation with methyl bromide (MeBr), to be heat treated, or kiln dried. Sulfuryl fluoride (SF) is a candidate fumigant to replace MeBr under certain conditions. SF fumigations were conducted in 432-liter Lexan chambers held in a 6.1-m (20-foot) refrigerated container for temperature control. Each fumigation consisted of 12 Populus spp. 10- by 10- by 115-cm timbers, of high moisture content, naturally infested with Asian longhorned beetle. During 2001, we fumigated wood for 24 h at a range of doses (20–112 g/m³) and temperatures (4.4, 10.0, 15.6, and 21.1°C) and subjected the data to probit analysis. Confirmatory fumigations were

conducted at doses of 120 and 104 g/m<sup>3</sup> at temperatures of 10.0 and 15.6 or 21.1°C, respectively, which resulted in complete kill of all larvae. Pupae that became available later in the year as temperatures

warmed were fumigated at 15.6 and 21.1 °C with  $104\,\mathrm{g/m^3}$ , which resulted in complete pupal mortality. The next year (2002), we conducted 24-h fumigations with doses of  $116\,\mathrm{g/m^3}$  at 4.4 and 10.0 °C with cold-harvested wood infested with cold-acclimated larvae. Cold-acclimated larvae required much higher concentration times time (CxT) product for control at 4.4 and 10.0 °C compared with nonacclimated larvae. Sulfuryl fluoride treatments at a dose of  $104\,\mathrm{g/m^3}$  and temperature of 15.6 °C and above and that achieved a CxT product of  $1,095\,\mathrm{g-h/m^3}$  or above are recommended for RWPM infested with Asian longhorned beetle larvae and pupae.

**KEY WORDS** fumigation, sulfuryl fluoride, quarantine

Methyl bromide (MeBr) is currently regulated internationally through acceptance of the Montreal Protocol of 1998 (Anon. 1998), and much research has been directed toward MeBr alternatives and the reduction of use of MeBr. Sulfuryl fluoride ( $SO_2F_2$ ; SF) is one of the most promising alternative fumigants to MeBr for controlling wood-boring insects. It has already proven to be successful against termites of several species (Su and Scheffrahn 1986) and penetrated dry wood well (Scheffrahn et al. 1992). Soma et al.

(1996) have shown SF to be effective against seven species of wood borers and bark beetles, although egg stages were more resistant than the other stages. Mizobuchi et al. (1996) had similar results with SF against five species of ambrosia beetles (Coleoptera: Scolytidae). Eggs of *Xyleborus pfeili* (Ratzeburg) required an estimated 24-h dose of >80 g/m³ for a lethal dose (LD)<sub>95</sub>. Furthermore, Soma et al. (1997) found that to achieve 100% mortality with eggs of *Monochamus alternatus* (Coleoptera: Cerambycidae), a 24-h dose estimated by probit analysis of 100 g/m³ SF at 25°C was required. This dose was five-fold greater than that required for either larvae or pupae.

More than 400 insects that feed on trees and shrubs have been introduced into the United States (Haack and Byler 1993, Mattson et al. 1994), primarily as a result of the use of solid wood packing in world trade. Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), the Asian longhorned beetle, is native to western and northern China (including Gansu Province) and Korea (Lingafelter and Hoebeke 2002) and was probably introduced into the United States in the 1980s. It was first found in New York in 1996 within

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solid wood packing materials (Haack et al. 1996, Cavey et al. 1998). The Asian longhorned beetle is a very damaging pest of *Populus* spp. trees in China (Li and Wu 1993). Asian longhorned beetle damages trees by first-third instars feeding on the cambium layer of the host tree, by later instars feeding in the xylem that is reached by tunnels created as the large larvae feed (Wang et al. 2000), and by the adults feeding on young twigs. Nowak et al. (2001) have estimated that as many as 1.2 billion urban shade trees could be potentially damaged, with a total value of \$669 billion, if the Asian longhorned beetle becomes established across the United States. Also, the Asian longhorned beetle is a potentially serious threat to American and Canadian hardwood forests, the lumber and maple sugar industries, tourism, the esthetic and dollar values of properties, and to the species diversity of the forest environment.

The Animal and Plant Health Inspection Service, Plant Protection and Quarantine (APHIS-PPQ) has in place treatment schedules (Schedule T-404-b-1-1 and T-404-b-1-2, normal atmospheric pressure (NAP) and vacuum fumigation, respectively) for the MeBr fumigation of regulated wood packing material (RWPM) in containers for control of wood-boring insects (Anon. 2004). PPQ fumigation schedules for phosphine and sulfuryl fluoride were recently rescinded due to fumigation failures discovered during the progress of the investigation reported here. Currently, methyl bromide fumigation and heat treatment (Schedule T-404-b-4, kiln drying) are the only treatments allowed for RWPM coming into the United States from China.

The preliminary fumigations conducted during 2000 indicated that the USDA schedule T-404-b-2 was not adequate for eradication of Asian longhorned beetle larvae in large, green timbers, and the schedule was subsequently modified to exclude wood-boring beetles.

We report here a series of fumigations to test or improve the USDA schedule for sulfuryl fluoride fumigation of RWPM for Asian longhorned beetle and other wood-boring insects.

### Materials and Methods

General Methods. During 2000, in the city of Huhehaote (Hu-he-hao-t'e), Inner Mongolia, Peoples' Republic of China, we conducted trial SF fumigations of timbers infested with Asian longhorned beetle larvae. The purpose was to develop techniques and to set approximate starting doses for more detailed fumigations to be conducted elsewhere the following years. Infested wood was obtained from local infested *Populus* spp. trees that were felled and sawn into 10- by 10-cm by 1.15-m-long timbers. Twelve pieces 10- by 10-cm square or similar sized quarter-round pieces of larger logs were debarked and used in each chamber. Fumigations were conducted at 4.4, 10.0, 15.7, and 21.1°C, with one replicate at several chosen doses. The logs had light infestation, so we artificially added field-

Table 1. Moisture content and amount of wood for fumigations conducted at four temperatures

Parameter		e content t basis <sup>a</sup>	Wood (kg)/fumigation					
	Square	Rounds	4.4°C	10.0°C	15.6°C	21.1°C		
$\overline{n}$	5	5	12	12	11	11		
Mean	40.06	27.1	71.85	70.43	66.17	66.82		
SE	1.938	1.231	1.251	0.732	1.260	0.778		
Sample variance	18.78	7.58	18.77	6.43	17.45	6.66		
Min.	33.8	23.6	66	66.5	62	63.5		
Max	44.4	31.2	78	73.6	71.6	70		
0.95 CI	5.38	3.42	2.75	1.61	2.81	1.73		

"Moisture content wet basis according to ASTM Procedure D-4442-92 Method A (Anon. 1997).

collected larvae, collected locally, by drilling six 0.95-cm-diameter holes to the center of each of six timbers and inserting a larva in each hole. These larvae were collected at another site in Gansu Province. Each hole was then partially filled with sawdust and plugged with a maple dowel. This guaranteed at least 36 larvae per fumigation.

All fumigations were conducted in chambers constructed of 0.9525-cm (3/8-in.)-thick Lexan and that measured 0.61 m in height by 0.61 m in width by 1.23 m in length and had an internal volume of 432 liters. Each chamber was fitted with two  $\approx 6.35$ -mm (0.25-in.) stainless steel ball valves with hose barbs used to draw a vacuum, introduce the fumigants, and monitor concentrations. Each chamber was equipped with a 12-V DC cooling fan for fumigant mixing. Six chambers were contained in each of two 6.1-m (20-foot)-long refrigerated cargo containers capable of holding set point  $\pm 0.1$ °C. After 24 h, the chambers were opened and aerated  $\approx 30$  min by the use of the reefer's large air exchange capacity when doors were opened.

To conduct fumigations, sufficient wood was added to each chamber to give a wood volume load factor of  $\approx$ 25–27%. The volume of wood affects fumigant sorption and available headspace and therefore must be kept reasonably constant. Wood loads for each chamber were weighed and were approximately equal among replicates. Three layers of wood were loaded and separated by 8-mm-thick by 34-mm-wide common wood lath spacers. Internal wood temperatures were monitored by inserting type T thermocouples into holes drilled into the center of at least two timbers, the holes of which were then plugged with rubber putty. When the wood reached the desired internal temperature (4.4, 10.0, 15.6, or 21.1°C), the chamber lids were put on and sealed all around with a layer of reinforced, plastic duct tape (Tape-it, Brooklyn, NY). The fumigant doses were computed volumetrically by calculating the volume of the required grams of MeBr gas with the V = nRT/P relationship (the ideal gas law), that compensated for ambient gas temperature and ambient atmospheric pressure at ≈0.85 atmospheres (640 mmHg). The calculated amount of gas was then transferred to 10-liter gas bags. To introduce the fumigant, a slight vacuum was cre-

Table 2. Probit analysis of dose-mortality data for A. glabripennis after 24-h fumigation with sulfuryl fluoride at four temperatures

Temp (°C)	Slope	Intercept	n	df	$\chi^2$	Hetero.	Probits	Control level (%)	Estimated dose (g/m³)	0.95 CI
4.4										
Parameter	6.878	-6.051	1,791	9	49.58	5.51	7.3264	99	88.1	75.2-118.7
SE	0.508	0.906					8.0904	99.9	113.8	
							8.7191	99.99	140.4	
							9.0000	99.99683	154.3	115.6-290.2
10.0										
Parameter	7.128	-5.839	1,552	9	43.57	4.84	7.3264	99	70.3	60.6-89.7
SE	0.408	0.662					8.0904	99.9	90.0	
							8.7191	99.99	110.3	
							9.0000	99.99683	120.7	93.6-188.3
15.6										
Parameter	7.079	-5.284	1,802	8	51.73	6.47	7.3264	99	60.5	51.5-80.4
SE	0.375	0.576					8.0904	99.9	77.5	
							8.7191	99.99	95.1	
							9.0000	99.99683	104.2	78.8-175.3
21.1										
Parameter	8.675	-7.852	1,963	8	126.3	15.79	7.3264	99	56.2	46.7-100.8
SE	0.536	0.841					8.0904	99.9	68.8	
							8.7191	99.99	81.3	
							9.0000	99.99683	87.6	63.3-276.5

ated with a vacuum pump and measured with an open arm manometer until pressure was  $\approx 80-100$  mmHg below ambient pressure. The gas bag was then attached and the valve opened, thus allowing the gas to quickly flow into the chamber. After all gas was infused, the valve was left open only long enough to equalize any remaining pressure differential. Chamber fans were turned on for 0.5 h to achieve uniform fumigant concentrations.

Fumigant concentrations in test chambers and untreated control chambers were monitored at intervals of 0.5, 2, 4, 8, 12, and finally 24 h after gas introduction, using a thermal conductivity (TC) instrument, the XK-III, of Chinese manufacture (CPQ Technology Company, Animal and Plant Quarantine Institute, Beijing, People's Republic of China) and was functionally similar to the American made Fumiscope (Key Chemical, Clearwater, FL). We typically conducted five

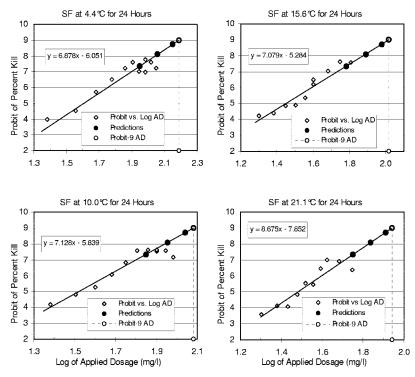


Fig. 1. Regression of probits of percentage of kill versus log of applied dose of SF. Predicted points are for 99.0, 99.9, 99.99 and Probit-9 level of control.

Table 3	Probit analysis of CxT	mortality data for A	olahrinennis after	24-h fumigation w	ith sulfuryl fluoride	at four temperatures

Temp (°C)	Slope	Intercept	n	df	$\chi^2$	Hetero.	Probits	Control level (%)	Estimated dose (g-h/m³)	0.95 CI
4.4										
Parameter	5.7450	-10.401	1,791	9	26.00	2.89	7.3264	99	1,218.2	1,058.3-1,474.1
SE	0.2790	0.75743					8.0904	99.9	1,654.6	
							8.7191	99.99	2,128.8	
							9.0000	99.99683	2,382.4	1,888.8-3,316.4
10.0										
Parameter	5.8880	-9.969	1,552	9	77.52	8.61	7.3264	99	886.0	706.5-1,473.1
SE	0.5060	1.392					8.0904	99.9	1,195.7	
							8.7191	99.99	1,530.2	
							9.0000	99.99683	1,708.5	1,106.8-7,916.2
15.6										
Parameter	5.7960	-8.464	1,802	8	42.44	5.30	7.3264	99	530.1	440.9-722.3
SE	0.3070	0.741					8.0904	99.9	718.1	
							8.7191	99.99	921.8	
							9.0000	99.99683	1,030.7	749.6-1,800.8
21.1									,	
Parameter	7.1360	-10.397	1,963	8	15.00	1.87	7.3264	99	304.6	276.7-349.0
SE	0.4640	1.059	,				8.0904	99.9	389.7	
							8.7191	99.99	477.3	
							9.0000	99.99683	522.6	436.8-682.7

treatments of selected doses and one control fumigation in each container. Because the wood was green, and  $CO_2$  was evolved from green wood, we also measured the  $CO_2$  with an infrared gas analyzer of Chinese manufacture (Beijing Computer Application Institute, Beijing, China). The instrument was calibrated by the manufacturer with 10%  $CO_2$  with nitrogen balance. Because  $CO_2$  is additive to SF when using a thermal conductivity type of instrument, the actual SF

concentration was deduced by correlating the control  $\mathrm{CO}_2$  reading with the control TC readings obtained from two control chambers loaded with a comparable supply of wood and then subtracting this value from the test chamber readings taken at the same time.

After fumigation, the wood was stacked and covered with a woven polypropylene tarp for a postfumigation holding period of 6–8 d. After this time, the wood was carefully split open with sledge and chisel,

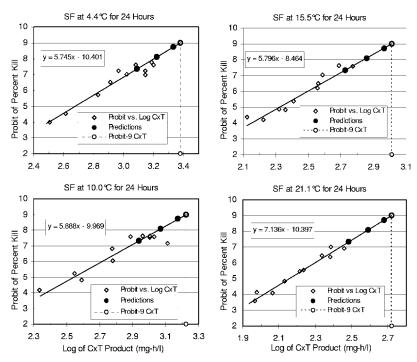


Fig. 2. Regression of probits of percentage of kill versus log of CxT product. Predicted points are for 99.0, 99.9, 99.99, and Probit-9 level of control.

Table 4. Results from 24-h sulfuryl fluoride confirmatory fumigation for Asian longhorned beetle in 10- by 10-cm timbers at several doses, with CxT product calculated for each dose

Temp (°C)/ dose $(g/m^3)$		SF co	nen (g/m³)	at hour		${\rm CxT} \atop {\rm (g-h/m^3)}$	Asian longhorned beetle larvae		C. cossus	
	0.5	2	4	12	24		No.	% dead	No.	% dead
4.4/160	220		151.3	74.2	43.58	2,368	253	14.6	3	100
$\pm$ SD	n/a		$\pm 16.5$	$\pm \ 8.1$	$\pm 7.7$	± 222	185 pupae	92.5		
4.4/136	172	129.4	101.9	49.9	28.3	1,620	781	98.2	5	100
$\pm$ SD	n/a	$\pm 2.91$	$\pm 4.59$	$\pm 5.36$	$\pm \ 4.71$	$\pm 106.1$				
10.0/120	146.0	111.4	87.4	38.2	16.2	1,294	726	100	4	100
$\pm$ SD	$\pm 1.5$	$\pm 3.2$	$\pm 2.9$	$\pm 3.4$	$\pm 2.5$	$\pm 68.5$				
15.6/104	115.1	89.8	73.1	34.8	13.5	1,095.6	539	100	8	100
$\pm$ SD	$\pm 2.7$	$\pm 4.0$	$\pm 5.6$	$\pm 6.6$	$\pm 5.7$	$\pm 136.2$	22 pupae	100		
21.1/104	115.3	88.1	68.7	29.9	8.8	993.5	473	100	6	100
$\pm$ SD	$\pm 6.4$	$\pm \ 5.8$	$\pm 7.4$	$\pm  6.8$	$\pm \ 5.0$	$\pm 149.2$	20 pupae	100		

There were five replicates per dose. Larvae of the lepidopteran species *C. cossus* also were found within wood. n.a., not applicable.

and all larvae were counted and evaluated. Larvae were considered dead if they were limp and showed no movement. Larvae that were turgid or had body movement were considered alive. Moribund larvae that were completely limp, or discolored, and had only mandibular movement after 12–14 d were considered dead

**2001 Fumigations.** In 2001, we moved operations to the city of Lanzhou, Gansu Province, where natural, heavily infested wood was more available. Selected treatment wood used was commercially sawn from naturally infested *Populus* spp. logs to a dimension of 10 by 10 by 115 cm before use. Chambers were loaded with eight sawn pieces and four debarked logs of ≈10-12-cm diameter. All wood loads were weighed and kept approximately equal between fumigations. Chamber loading was with a 25-27% load factor. We conducted a series of fumigations at temperatures of 4.4, 10.0, 15.6, and 21.1°C at selected doses estimated to give levels of control suitable for probit analysis. Based on the results, we conducted a series of confirmatory fumigations at selected doses. Five replicates per dose were fumigated, and there was one untreated control. Readings of CO2 were recorded and correlated with XK-III readings with the control chambers. The CO<sub>2</sub> component was then deducted from the XK-III SF readings to adjust to true value.

Table 5. Moisture content and amt of cold-harvested wood per fumigation

Parameter		e content t basis <sup>a</sup>	Wood (kg)/fumigation				
	4.4°C	10.0°C	4.4°C	10.0°C	10.0°C		
${n}$	4	8	12	12	6		
Mean	51.1	52.2	103.1	102.9	92.0		
SE	0.87	1.98	0.14	0.19	0.18		
Range	4.2	15.54	2	2.5	1		
Min.	49.18	42.51	102	101.5	91.5		
Max	53.38	58.05	104	104	92.5		
0.95 CI	2.78	4.69	0.30	0.42	0.47		

<sup>&</sup>lt;sup>a</sup> Moisture content wet basis according to ASTM Procedure D-4442-92 Method A 9 (Anon. 1997).

Five samples of sawn and round wood each were selected for moisture determination. Slices several cm thick from the center of each length were tested for wet basis moisture content in an air oven according to ASTM procedure D-4442-92 (Anon. 1997).

2002 Fumigations. During 2002, we conducted a series of fumigations, following procedures as described above, by using wood that was harvested during the cold season, so that the wood temperatures were never allowed to exceed 10.0°C before fumigation. This was done to ascertain whether Asian long-horned beetle larvae acclimated to cold temperatures were more tolerant to fumigation at the lower temperatures. In previous tests (2001), wood needed to be chilled from warmer temperatures, where larvae were once physically active.

Statistical Analysis. For 2001 and 2002 fumigations, results were tabulated and analyzed using the Polo Plus software program (Robertson et al. 2002). Plots of the Probit regression and statistical summaries were made using Microsoft Excel (Anon. 2000). POLO reported the Probit-9 response as Probit-4. To achieve the commonly accepted designation, 5 was added to the intercept of each regression analysis. Probit analysis was used to analyze mortality as it related to applied dose and accumulated CxT (concentration times time) product.

2003 Fumigations. During 2003, we conducted a series of fumigations to ascertain the ability of fumigated larvae to recover if they were provided with artificial diet. Fumigations were conducted as described previously, and at four temperatures (4.4, 10.0, 15.6, and 21.1°C). Larvae were classified as alive if there was body movement, and the larvae were turgid. Larvae were considered moribund if the body was limp with only mandibular movement. A collective total of 178 surviving larvae, 100 moribund larvae, and live 50 control larvae were individually placed in 177-ml (6-liquid ounce) plastic cups with artificial diet (Payne et al. 1975) and observed daily for 12–16 additional days to look for activity and determine whether moribund larvae would recover.

Table 6.	Probit analysis of applied dose and CxT mortality data for A. glabripennis after 24-h fumigation with sulfuryl fluoride at 4.4
and 10.0°C	

Temp (°C)	Slope	Intercept	n	df	$\chi^2$	Hetero.	Probit	Control level (%)	Estimated dose (g/m³)	0.95 CI
4.4	Appl	ied dose								
Parameter	4.386	-2.175	2,301	7	65.986	9.4265	7.3264	99	146.66	112.0-239.5
SE	0.193	0.337					8.0904	99.9	219.02	
							8.7191	99.99	304.67	
							9.0000	99.99683	353.09	221.4-856.9
4.4	CxT	product								
Parameter	3.7450	-5.425	2,301	7	53.9920	7.7131	7.3264	99	2,540.5	1,900.1-4,187.1
SE	0.1670	0.488					8.0904	99.9	4,063.7	
							8.7191	99.99	5,981.3	
							9.0000	99.99683	7,108.9	4,280.2-17,651.0
10.0	Appl	ied dose								
Parameter	5.196	-3.447	3,058	12	143.12	11.927	7.3264	99	118.41	96.5-164.5
SE	0.18	0.307					8.0904	99.9	166.13	
							8.7191	99.99	219.50	
							9.0000	99.99683	248.60	175.9-443.0
10.0										
	CxT	product								
Parameter	4.4500	-6.656	3,058	12	60.5240	5.0436	7.3264	99	1,387.1	1,173.5-1,738.1
SE	0.1520	0.412					8.0904	99.9	2,059.7	
							8.7191	99.99	2,851.5	
							9.0000	99.99683	3,297.6	2,486.8-4,873.7

The larvae were in Populus spp. timbers sawn from naturally infested logs harvested in the Lanzhou, China, area during cold weather.

#### **Results and Discussion**

General Methods. High mortality due to extraction of larvae from the naturally infested wood, possible damage during the insertion process, and small numbers of larvae led us to abandon this approach. Instead, better quality (highly infested) naturally infested

wood sawn to uniform dimensions (10 by 10 cm by 1.15 m) was used in subsequent tests. The gas introduction and monitoring techniques were acceptable.

2001 Fumigations. The moisture content and wood load for fumigations conducted during 2001 are presented in Table 1. The wood was of high moisture

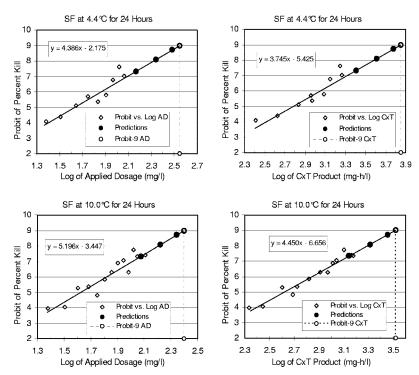


Fig. 3. Regression of probits of percentage of kill versus log of AD and CxT product. Wood was harvested cold and not subject to warming above 10°C.

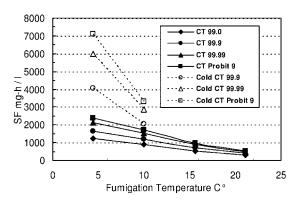


Fig. 4. Summary plot of CxT needed for control levels of 99.0, 99.9, 99.99%, and Probit-9 (99.99683%) for chilled or warmed wood, and winter harvested cold wood with cold adapted Asian longhorned beetle larvae.

content, as high as 44.4% wet basis. Typical wood load weights were between ≈66 and 78 kg per chamber. Lighter weights were due to moisture loss as the wood dried. The result of probit analysis of applied dose is presented in Table 2 and Fig. 1. The result of probit analysis for CxT product is presented in Table 3 and Fig. 2. Lower temperatures required higher doses to meet the estimated level of Probit-9 (99.99683%) control. This dose was only estimated, because maximum numbers of 1,552-1,936 larvae were found in a series of fumigations at each temperature. The result of the series of confirmatory fumigations based on probit analysis is presented in Table 4. It can be seen that the dose to give estimated Probit-9 kill at 4.4°C did not kill all larvae, but at 10°C and above all larvae and pupae were killed. Numerous Cossus cossus (L.) (Lepidoptera: Cossidae) larvae were found in the wood upon postfumigation evaluation. All were killed. C. cossus is not a pest of quarantine significance in the United States. We point out that doses significantly above 120 g/m<sup>3</sup> are difficult to apply with tarpaulin fumigation with a single dose due to the volume of the fumigant needed.

2002 Fumigations. The moisture content and wood loading for fumigations conducted with cold wood are shown in Table 5. It was observed that at 15°C, larvae were actively feeding and producing borings. With

Table 7. Survivorship of live and moribund larvae placed on artificial diet for  $14{-}16$  d after  $24{-}h$  sulfuryl fluoride fumigation at doses of  $20{-}96$  g/m $^3$ 

Fumigation	Live la	rvae <sup>a</sup>	Moribund larvae $^b$		
temp (°C)	Count	Live	Count	Live	
4.4	75	61	44	0	
10.0	32	24	15	0	
15.6	25	20	32	2	
21.1	46	42	9	0	
Total	178	147	100	2	
All controls	50	49			

<sup>&</sup>lt;sup>a</sup> Live larvae, with body movement or turgid body.

Table 8. Proposed treatment schedule for 24-h sulfuryl fluoride fumigation of RWPM in cargo containers for control of woodboring insects

Temp	Dose		Min. con	en (g/m <sup>3</sup> )	at hour	
(°C)	$(g/m^3)$	0.5	2	4	12	24
≥16	104	115	90	73	34	14

experiments during 2001, we needed to chill wood to 4.4 and 10.0°C so wood could be test fumigant efficacy at these temperatures. In practice, fumigations would be carried out at ambient temperatures or with warmed wood. Therefore, fumigations conducted with originally cold, unchilled wood had dormant larvae or larvae acclimated to cold temperatures. It was thought that cold-acclimated larvae would be more difficult to kill in unwarmed, higher moisture wood. The results in Table 6 and Fig. 3 indicate this is the case. The predicted dose and CxT for control at 4.4°C of apparently cold acclimated larvae in green wood was high (353 g/m<sup>3</sup> and 7108.9 g-h/m<sup>3</sup>, respectively). The same was true at 10.0°C. A comparison of the relative CxT required to gain levels of control from 99.0% to Probit-9 (99.99683%) is illustrated in Fig. 4. Supporting this, Zachariassen (1973) reported that the winter osmality of hemolymph of the adult cerambycid Rhagium inquisitor L. was ≈10 times the summer concentration and that the freezing point of about -6°C was far lower than for many other cold-acclimated insects. It is not known whether the same physiology applies to overwintering Asian longhorned beetle larvae, but the result suggests a very low metabolic condition in such larvae.

2003 Fumigations. The data presented in Table 7 indicate that our method of assessing larval mortality was accurate. Control larvae had 49 of 50 survivors on diet, and 147 of 178 fumigated survivors survived on the diet. All but two moribund larvae from doses of 24–96 g/m³ fumigations at all temperatures failed to recover after 10–12 d postfumigation holding and an additional 12–16 d on artificial diet. The two moribund larvae that recovered were from the nine larvae from the second lowest dose of  $32~\mathrm{g/m^3}$ . This low dose will not be found during a normal fumigation.

Although eggs of wood-boring cerambycids and scolytids were more tolerant of SF than all other stages (Soma et al. 1996, 1997; Mizobuchi et al. 1996), we must point out that the higher CxT exposure required for naked eggs, and likely eggs just under the surface, will be achieved in actual fumigation. The CxT required for larvae deep in the wood would exceed the CxT required for naked eggs, due to the high doses and time required for such doses to penetrate into timbers. Confirmatory tests with very large numbers of larvae to absolutely confirm Probit-9 mortality are unlikely to occur, because that would require ≈31,500 larvae per test, a totally unrealistic number for this Cerambycidae species, and a number not expected to occur in international shipments.

The results of this research support a fumigation schedule with SF for Asian longhorned beetle larvae

 $<sup>^{\</sup>it b}$  Moribund larvae, with limp body and only mandibular movement.

and pupae, as shown in Table 8. We recommend that this schedule support an alternative to MeBr at 16°C and above and be considered for inclusion under Rule 15 (Anon. 2002).

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